



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/CA97/00176 (22) International Filing Date: 17 March 1997 (17.03.97) (71)(72) Applicants and Inventors: TAM, Yun, K. [CA/CA]; 2012 104A Street, Edmonton, Alberta T6J 5L8 (CA). ZAMAN, Nuzhat [BD/CA]; Apartment #304, 12325 Lansdowne Drive, Edmonton, Alberta T6H 4L4 (CA). (72) Inventor; and (75) Inventor/Applicant (for US only): ISHIDA, Naobumi [JP/CA]; 11419 24 Avenue, Edmonton, Alberta T6J 4Z1 (CA). (74) Agent: WALTER, Robert, H.; G. Ronald Bell & Associates, P.O. Box 2450, Station D, Ottawa, Ontario K1P 5W6 (CA).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: COMPOSITION FOR PREVENTION OF HEPATIC STEATOSIS (57) Abstract <p>Prolonged treatment with parenteral nutrition tends to induce hepatic steatosis, otherwise known as fatty liver. Increased formation of toxic secondary bile acids caused by prolonged contact of bile acids with intestinal bacteria, and reabsorption of these secondary bile acids from the intestinal lumen may have toxic effects on the liver resulting in hepatic steatosis. It has been discovered that bile acid sequestrants conventionally used for lowering serum cholesterol, can prevent or mitigate the hepatic steatosis associated with parenteral nutrition. Oral ingestion of a bile acid sequestrant, preferably cholestyramine, optionally in combination with an immunonutrient such as an omega-3 polyunsaturated fatty acid, a short-chain fatty acid, glutamine, arginine, an antioxidant, ribonucleic acids or nucleotides can prevent or mitigate hepatic steatosis. The invention also relates to prevention or mitigation of hepatic steatosis resulting directly or indirectly from other conditions such as cancer chemotherapy, sepsis, endotoxemia, burns, compromised intestinal function, bacterial translocation or AIDS.</p>		

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COMPOSITION FOR PREVENTION OF HEPATIC STEATOSIS

Technical Field

The invention relates to the field of liver treatment, and more particularly
5 to the use of a bile acid sequestrant for the purpose of preventing or mitigating
hepatic steatosis.

Background Art

The administration of nutrients through intravenous feeding via an
indwelling catheter, known as parenteral nutrition or total parenteral nutrition
10 (TPN), bypasses the digestive process and delivers the nutrients required by the
body, such as amino acids, sugars, fats, vitamins and minerals, directly into the
bloodstream.

Parenteral nutrition is widely used to provide nutrients to seriously ill
individuals who are unable to tolerate oral enteral feeding. However, bypassing
15 digestion through parenteral nutrition can lead to intestinal bacterial overgrowth,
bacterial translocation from the intestinal lumen into the body, and endotoxemia.
Parenteral nutrition can also lead to fat infiltration in the liver, known as hepatic
steatosis, hepatosteatosis or fatty liver.

A documented link exists between hepatic dysfunction, as an indicator of
20 liver damage, and parenteral nutrition (Fisher, *Gastroenterol. Clin. N. Am.*,
18:645-666, 1989; Hodes et al., *J. Pediatr. Surg.* 17:463-468, 1982). Hepatic
dysfunction presents a major problem in parenteral nutrition treatment of infants
and children. Hepatic dysfunction may be evidenced by elevated levels of serum
hepatic enzymes and bilirubin (Grant et al., *Surg. Gynecol. Obstet.* 145:573-580,

1977) or by the development of hepatic steatosis (Keim, *JPEN* 11:18-22, 1987; Keim et al., *J. Nutr.* 114:1807-1815, 1984).

Prolonged periods of parenteral nutrition therapy, during which patients are enterally fasted, results in reduced gastrointestinal motility, reduced
5 gastrointestinal transit time and a reduction in fecal output. These effects may be responsible for increased reabsorption of secondary bile acids from the intestinal lumen, thereby increasing the concentration of secondary bile acids in the serum (Vileisis et al., *J. Pediatr.* 96:893-897, 1980). Secondary bile acids, particularly lithocholic acid, formed by bacterial dehydroxylation of primary bile
10 acids in the distal small intestine or colon, have shown toxicity in a number of animal species (Yousef et al., *Gastroenterology* 80:233-241, 1981).

During parenteral nutrition treatment and enteral fasting, the intestine remains dormant and colonic bacteria are believed to have a greater opportunity to form secondary bile acids. The accompanying reduced intestinal motility can
15 extend the time during which secondary bile acids can be formed and reabsorbed through enterohepatic recycling. Thus, the toxic bile acid pool in the body can increase (Hofmann, *Hepatology* 4:4S-14S, 1984).

Cholestyramine is a basic anion exchange resin capable of binding bile acids, thus known as a bile acid sequestrant. Cholestyramine is conventionally
20 used for lowering blood cholesterol through interruption of enterohepatic recycling of bile acids. Compositions containing cholestyramine along with a serum lipid regulating agent are disclosed in U.S. Patent No. 4,814,354 for the purpose of blood cholesterol lowering. Compositions containing cholestyramine and a nonabsorbable nondigestible polyol polyester for use in lowering blood
25 cholesterol are disclosed in U.S. Patent No. 5,116,610.

When administered orally to a patient, cholestyramine remains protonated at the alkaline pH of the contents of the intestinal lumen thereby allowing maximum binding of bile acids to take place. The cholestyramine resin, thus having bile acids bound thereto, is then excreted from the body through the feces, thereby preventing or reducing reabsorption of the bound bile acids. Other bile acid sequestrants can produce similar results (Ast & Frishman, *J. Clin. Pharmacol.* 106:99-106, 1990). Cholesterol is a precursor molecule in bile acid synthesis. By eliminating bile acids from the body, replacement bile acids will be synthesized from the body's cholesterol pool, thus tending to lower blood cholesterol.

Other physiological conditions which may cause hepatic steatosis, include cancer chemotherapy, endotoxemia, sepsis, burns and various intestinal disorders, bacterial translocation, AIDS, or low birth weight in infants.

Some synthetic resins are effective in removing proteinaceous materials from biological fluids (Nolan et al., Effect of cholestyramine on endotoxin toxicity and absorption. *Digestive Diseases* 17:161-166, 1972). United States Patents Nos. 3,769,401 and 3,097,141 disclose processes for removing proteinaceous materials from biological fluids as a method of detoxification. These processes employ ionically charged resins for which proteinaceous material has affinity when placed in intimate contact therewith.

An object of the present invention is to prevent or mitigate hepatic steatosis which may result from parenteral nutrition treatment.

Disclosure of Invention

It has been discovered that compositions containing a bile acid sequestrant such as cholestyramine can prevent or mitigate hepatic steatosis associated with parenteral nutrition treatment or resulting from other treatments or conditions.

5 According to one aspect of the invention, there is provided a use of a bile acid sequestrant, characterized in that an effective amount of the sequestrant is ingested by a subject for prevention or mitigation of hepatic steatosis.

 According to another aspect of the invention, there is provided a use of a composition for prevention or mitigation of hepatic steatosis in a subject,
10 characterized in that the composition is adapted for oral or enteral administration and comprises an effective amount of a bile acid sequestrant and a pharmaceutically acceptable carrier.

 According to a further aspect of the invention, there is provided a composition adapted for oral or enteral administration to a subject for prevention
15 or mitigation of hepatic steatosis, characterized in that the composition comprises an effective amount of a bile acid sequestrant, an immunonutrient and a pharmaceutically acceptable carrier.

 According to a further aspect of the invention, there is provided a method of preventing or mitigating hepatic steatosis in a subject, characterized by the
20 ingestion by the subject of an effective amount of a bile acid sequestrant.

 According to another aspect of the invention, there is provided a method of preventing or mitigating liver damage in a subject, characterized by the ingestion by the subject of a composition comprising an effective amount of a bile acid sequestrant and a pharmaceutically acceptable carrier.

25 Hepatic steatosis may also result from other conditions. Thus, the invention may be used to prevent or mitigate hepatic steatosis ultimately

resulting from such conditions as cancer chemotherapy, endotoxemia, sepsis, burns, bacterial translocation and AIDS. The invention may also be used for prevention or mitigation of hepatic steatosis resulting from impaired intestinal function due to other specific or unknown factors.

5 The invention may be used to prevent or mitigate hepatic steatosis caused by factors of a specific or unknown origin.

Hepatic steatosis may also result from or result in a compromised immune system. The presence of immunonutrients in the composition of the invention may serve to enhance intestinal function, further reducing susceptibility to
10 bacterial translocation, endotoxemia and sepsis. When combined with a bile acid sequestrant such as cholestyramine, immunonutrients can further assist in prevention or mitigation of hepatic steatosis.

Brief Description of Drawings

In drawings which relate to the invention,

15 Figure 1 is a photomicrograph showing a liver from a rat which has received parenteral nutrition without cholestyramine treatment;

Figure 2 is a photomicrograph showing a liver from a rat which has received parenteral nutrition and treatment with cholestyramine; and

20 Figure 3 is a photomicrograph showing a liver from rat which has consumed rodent chow.

Best Mode for Carrying Out the Invention

According to a preferred embodiment of the invention, a composition containing cholestyramine and a pharmaceutically acceptable carrier is delivered orally to a subject, and the cholestyramine is administered at an effective level

not exceeding about 40 g/day, based on an adult body weight, thus preventing or mitigating hepatic steatosis resulting from parenteral nutrition delivery. A preferable range is 1 to 40 g/day of cholestyramine.

All dosage levels noted herein are based on an adult human body weight which may range from about 40 kg to 150 kg. It is understood that the invention is also contemplated for use in individuals with a body weight outside this range, in which case the dosage would normally be adjusted proportionately. Severe weight loss is often associated with, for example, chemotherapy, AIDS, sepsis, burns, and conditions which necessitate parenteral nutrition.

Optionally, one or more immunonutrients may be added to the composition containing cholestyramine and a pharmaceutically acceptable carrier. Immunonutrients are herein defined as those nutrients which have a beneficial effect on immune function, and include but are not limited to amino acids such as arginine and glutamine; antioxidants such as vitamins A, C, E, and β -carotene; omega-3 polyunsaturated fatty acids such as linolenic acid (18:2), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6); short-chain fatty acids such as acetic, propionic and butyric acids; triglycerides containing any of the omega-3 polyunsaturated fatty acids and/or short-chain fatty acids; and ribonucleic acids or nucleotides.

A preferable administration range for free form arginine is about 17 to 25 g/day. A preferable level for glutamine is an effective amount not to exceed about 60 g/day. A preferable daily dosage for glutamine is about 0.16 ± 0.02 g/kg body weight. A preferable range for omega-3 polyunsaturated fatty acid is about 1 to 4 g/day. For ribonucleic acid or nucleotides, a preferable level of administration is about 0.75 to 3 g/day.

Two useful bile acid sequestrants are cholestyramine and colestipol (Ast & Frishman, *J. Clin. Pharmacol.* 106:99-106, 1990). Preferably, cholestyramine is used as the bile acid sequestrant. Cholestyramine is a synthetic, strongly basic anion exchange resin containing quaternary ammonium functional groups which are attached to a styrene-divinylbenzene copolymer (Merck Index, 9th Edition, 1976, p.2194).

Colestipol, often provided as the powder colestipol hydrochloride, is a basic anion exchange copolymer made up of diethylenetriamine and 1-chloro-2,3-epoxypropane (optionally hydrochloride) with approximately one out of every five amine nitrogens being protonated (Merck Index, 9th Edition, 1976, p.2436). The preferable level of administration is an effective amount not exceeding 30 g/day and a preferable range is about 5 to 30 g/day.

It is understood that the invention contemplates use of bile acid sequestrants in any dosage form or with any structural modification of the resin which does not interrupt efficacy of bile acid binding.

Various dosage forms of the bile acid sequestrant, consistent with any pharmaceutically acceptable dosage form can be used in the invention. Ingestion of the bile acid sequestrant or the composition of the invention either through oral administration in a primarily solid, gel or liquid form, or through an enteral feeding tube is contemplated by the invention.

The invention is preferably applied during the occurrence of the treatment or condition responsible for the liver damage.

The following example illustrates the invention.

Example

Rats weighing 200 - 230 g were anaesthetized with methoxyflurane and catheterized via the right jugular vein with a silicone rubber tube. The catheter

was tunnelled subcutaneously and exteriorized in the scapular region where it was connected to a coiled metallic spring mounted on a swivel to permit free mobility of the animal. After the surgery, each animal was placed in a metabolic cage in a room with a 12-hour light/dark cycle. Animals were
5 allowed to recover from surgery for at least 2 days during which they were given free access to standard laboratory rodent chow and water.

Animals were randomly assigned to one of three treatment groups: (a) PN, those receiving an infusion of parenteral nutrition; (b) PNC, those receiving an infusion of parenteral nutrition plus a daily oral dose of cholestyramine
10 (Questran™, Bristol-Myers) at a level of 0.34 g/kg body weight; and (c) CF, those receiving a parenteral infusion of saline and allowed free access to rodent chow.

All animals received infusions for either 7 or 14 days at a rate of 3 mL/hour, delivered by a volumetric infusion pump. PNC animals were
15 administered cholestyramine daily through oral gavage with a feeding needle. PN and CF animals were administered a corresponding volume of water daily through oral gavage with a feeding needle.

The parenteral nutrition solution comprised: 242 g/L dextrose, 52 g/L amino acids supplied in the form of 10% Travasol™ blend B with electrolytes
20 (Baxter Corporation, Canada), 2 mL/L of a multivitamin solution and 2.25 mmol/L of calcium gluconate.

At the end of a seven day experimental period, total serum bilirubin (mg/dL) was higher in the PN vs the PNC animals (PN, 0.62 ± 0.22 vs PNC, 0.36 ± 0.13 , $p < 0.05$, means \pm SD) although neither group differed
25 significantly from the CF animals (CF, 0.53 ± 0.36).

Serum amino acid levels were similar among the three treatment groups (Table 1). Elevated levels of serine, glycine, threonine and methionine were found in the PN animals as compared to the CF animals. Elevated levels of threonine and glycine were found in the PN vs the PNC animals.

5

Table 1
Serum Amino Acid Concentrations ($\mu\text{mol/L}$)

Amino Acid	Parenteral Nutrition (PN)	Parenteral Nutrition plus Cholestyramine (PNC)	Rodent Chow (CF)
Aspartic acid	18 \pm 15	13 \pm 2	31 \pm 25
Glutamic acid	88 \pm 46	66 \pm 17	74 \pm 40
Asparagine	59 \pm 35	67 \pm 22	76 \pm 41
Serine	137 \pm 93 ¹	244 \pm 69	203 \pm 94
Glutamine	548 \pm 228	507 \pm 113	436 \pm 108
Histidine	100 \pm 34 ¹	93 \pm 34	64 \pm 16
Glycine	572 \pm 177 ²	328 \pm 94	304 \pm 74
Threonine	354 \pm 103 ³	219 \pm 47	91 \pm 135
Citrulline	46 \pm 19	43 \pm 18	54 \pm 28
Arginine	188 \pm 68	186 \pm 63	189 \pm 86
Taurine	442 \pm 412	249 \pm 83	140 \pm 84
Alanine	705 \pm 526	316 \pm 141	546 \pm 207
Tyrosine	98 \pm 55	90 \pm 21	76 \pm 28
Tryptophan	75 \pm 33	62 \pm 25	51 \pm 31
Methionine	101 \pm 31 ¹	61 \pm 18	58 \pm 36
Valine	190 \pm 101	166 \pm 98	161 \pm 90
Phenylalanine	98 \pm 44	84 \pm 31	54 \pm 29
Isoleucine	90 \pm 44	75 \pm 35	93 \pm 51
Leucine	135 \pm 87	122 \pm 72	136 \pm 66
Ornithine	156 \pm 147	91 \pm 28	104 \pm 40
Lysine	637 \pm 365	563 \pm 186	809 \pm 241

Values are means \pm SD (n=6 in each group);

¹p < 0.05, different vs CF group;

²p < 0.05, different vs CF and PNC groups;

³p < 0.05, different vs PNC group.

Liver weight as a percentage of final body weight was lower in PN and PNC animals, compared with CF animals (PN, 3.34 ± 0.33 ; PNC, 3.41 ± 0.29 vs CF, 3.86 ± 0.30 ; $p < 0.05$, means \pm SD). Hepatic steatosis scores after 14 days of treatment were determined histologically, and although hepatic steatosis was absent in both the CF animals and the PNC animals, hepatic steatosis was observed in animals in the PN group.

Figure 1 depicts a representative photomicrograph of a rat liver from the parenteral nutrition (PN) group of animals. Hepatic steatosis is evident in the form of both large and small fat droplets (indicated by the symbol "F") throughout the hepatocytes.

Figure 2 depicts a representative photomicrograph of a rat liver from the parenteral nutrition plus cholestyramine (PNC) group of animals. Unlike Figure 1, a normal cellular architecture is evident and there are no signs of hepatic steatosis.

Figure 3 depicts a photomicrograph of a rat liver from the rodent chow (CF) group of animals, showing a normal cellular architecture similar to that of Figure 2.

Figures 1 to 3 were obtained from histological sections stained with H&E, and visualized at an original magnification of x 880.

Histological evidence illustrates that parenteral nutrition treatment is associated with hepatic steatosis as seen in Figure 1 and that cholestyramine treatment reversed the hepatoabnormality as evident from Figure 2. This result is consistent with the effect of cholestyramine in absorption of toxic secondary bile acids, since these bile acids contribute significantly to development of liver malfunction.

This example shows that oral administration of cholestyramine prevents hepatic steatosis induced by parenteral nutrition.

Industrial Applicability

The present invention allows for prevention or mitigation of the hepatic
5 steatosis normally associated with conditions such as parenteral nutrition, cancer
chemotherapy, endotoxemia, sepsis, burns, and other conditions which may
compromised intestinal function, bacterial translocation and AIDS. The use of a
bile acid sequestrant, optionally in combination with immunonutrients will result
in the reduction or minimization of hepatic steatosis and improved overall health
10 of the individual.

CLAIMS

1. A use of a bile acid sequestrant, characterized in that an effective amount of said sequestrant is ingested by a subject for prevention or mitigation of hepatic steatosis.
2. A use according to claim 1, characterized in that said sequestrant is cholestyramine.
3. A use of a composition for prevention or mitigation of hepatic steatosis in a subject, characterized in that the composition is adapted for oral or enteral administration and comprises an effective amount of a bile acid sequestrant and a pharmaceutically acceptable carrier.
4. A use according to claim 3, characterized in that the sequestrant is cholestyramine.
5. A use according to claim 1, 2, 3 or 4, characterized in that the hepatic steatosis is a result of a condition selected from the group comprising: parenteral nutrition, cancer chemotherapy, endotoxemia, sepsis, burns, compromised intestinal function, bacterial translocation and AIDS.
6. A use according to claim 5, characterized in that the hepatic steatosis is a result of parenteral nutrition.
7. A composition adapted for oral or enteral administration to a subject for prevention or mitigation of hepatic steatosis, characterized in that the

composition comprises an effective amount of a bile acid sequestrant, an immunonutrient and a pharmaceutically acceptable carrier.

8. A composition according to claim 7, characterized in that the sequestrant is cholestyramine.
9. A composition according to claim 8, characterized in that cholestyramine is included in the composition in an amount such that intake does not exceed about 40 g/day.
10. A composition according to claim 7, 8 or 9, characterized in that the immunonutrient is selected from the group comprising: amino acids, antioxidants, omega-3 polyunsaturated fatty acids, short-chain fatty acids, and triglycerides thereof.
11. A composition according to claim 7, 8, or 9, characterized in that the immunonutrient is ribonucleic acid or a nucleotide.
12. A composition according to claim 10, characterized in that the immunonutrient is selected from the group comprising: arginine, glutamine, vitamin A, vitamin C, vitamin E, β -carotene, linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and triglycerides thereof.
13. A composition according to claim 12, characterized in that the immunonutrient is arginine and is supplied at a level of about 17 to 25 g/day.

14. A composition according to claim 12, characterized in that the immunonutrient is glutamine and is supplied in an amount such that intake does not exceed about 60 g/day.
15. A composition according to claim 12, characterized in that the immunonutrient is an omega-3 polyunsaturated fatty acid and is supplied at a level of from about 1 to 4 g/day.
16. A use of a composition according to claim 7, 8, 9, 12, 13, 14 or 15, characterized in that the hepatic steatosis is a result of a condition selected from the group comprising: parenteral nutrition, cancer chemotherapy, endotoxemia, sepsis, burns, compromised intestinal function, bacterial translocation, or AIDS.
17. A use according to claim 16, characterized in that the hepatic steatosis is a result of parenteral nutrition.
18. A method of preventing or mitigating hepatic steatosis in a subject characterized by administration of an effective amount of a composition according claim 7, 8, 9, 12, 13, 14 or 15.
19. A method according to claim 18, characterized in that the hepatic steatosis is a result of a condition selected from the group comprising: parenteral nutrition, cancer chemotherapy, endotoxemia, sepsis, burns and compromised intestinal function, bacterial translocation, and AIDS.

20. A method of preventing or mitigating hepatic steatosis in a subject, characterized by the ingestion by the subject of an effective amount of a bile acid sequestrant.
21. A method according to claim 20, characterized in that the sequestrant is cholestyramine.
22. A method of preventing or mitigating hepatic steatosis in a subject, characterized by the ingestion by the subject of a composition comprising an effective amount of a bile acid sequestrant and a pharmaceutically acceptable carrier.
23. A method according to claim 22, characterized in that the sequestrant is cholestyramine.
24. A method according to claim 20, 21, 22 or 23, characterized in that the hepatic steatosis is a result of a condition selected from the group comprising: parenteral nutrition, cancer chemotherapy, endotoxemia, sepsis, burns, compromised intestinal function, bacterial translocation, and AIDS.
25. A method according to claim 19 or 24, characterized in that the hepatic steatosis is a result of parenteral nutrition.

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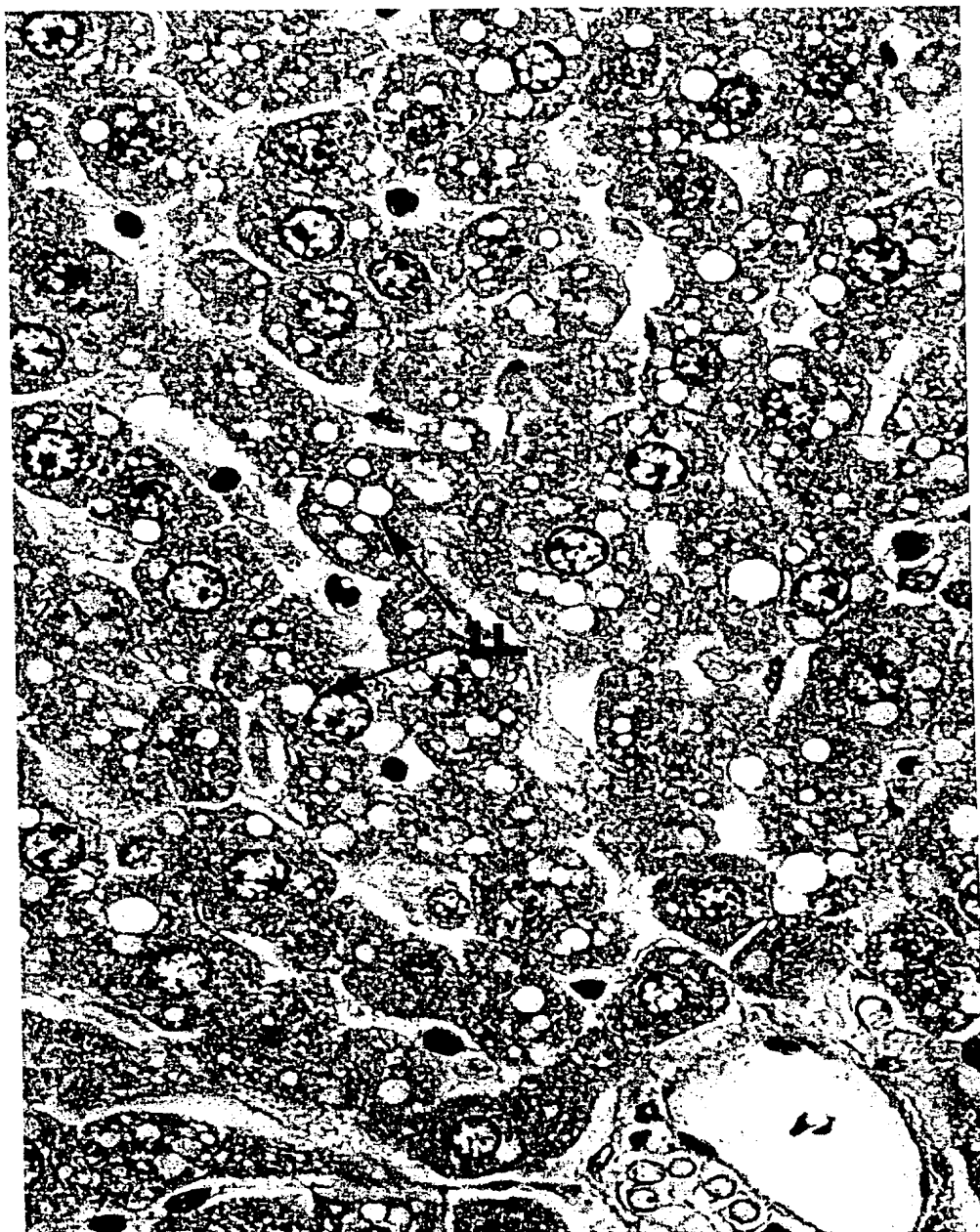


Figure 1

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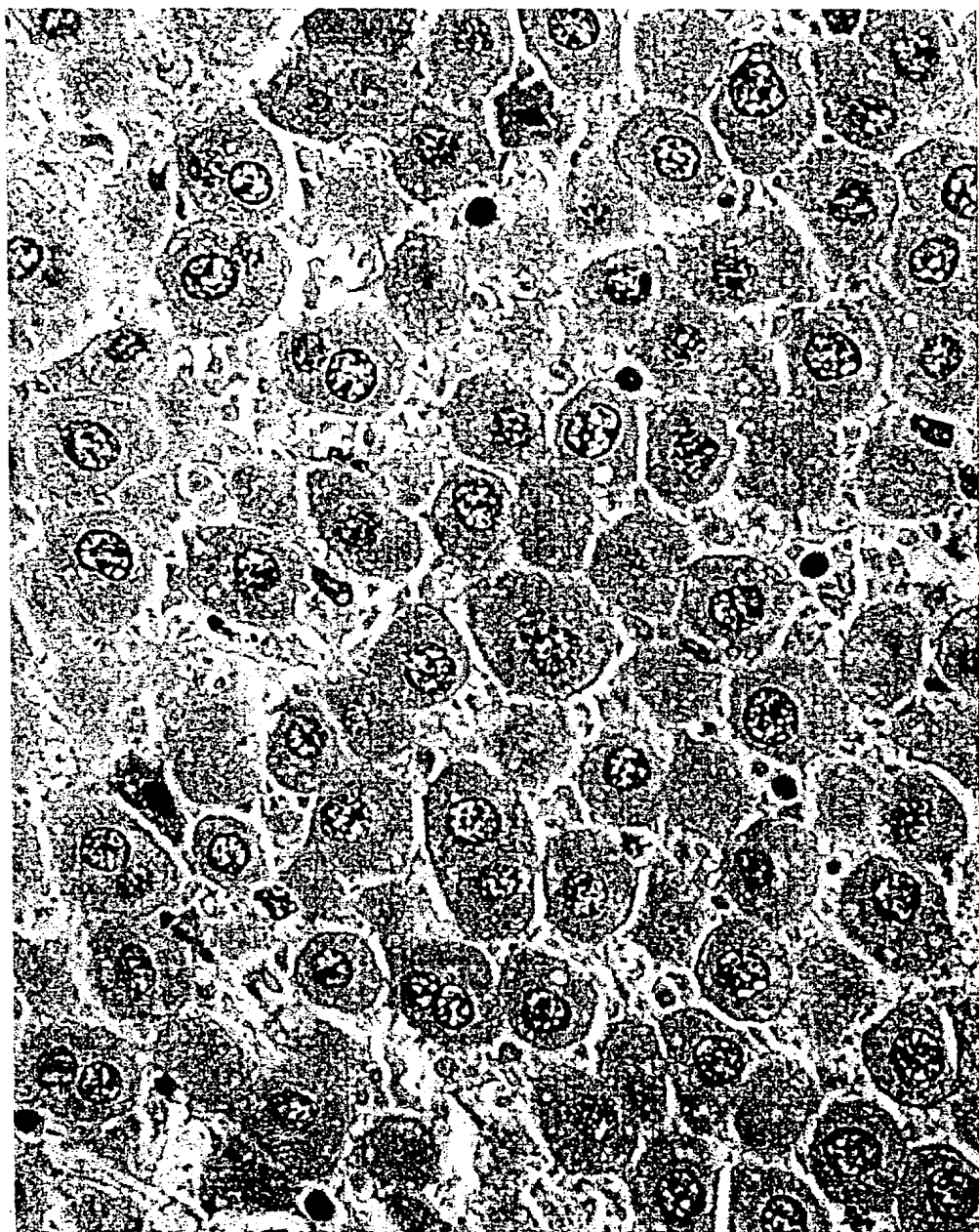


Figure 2

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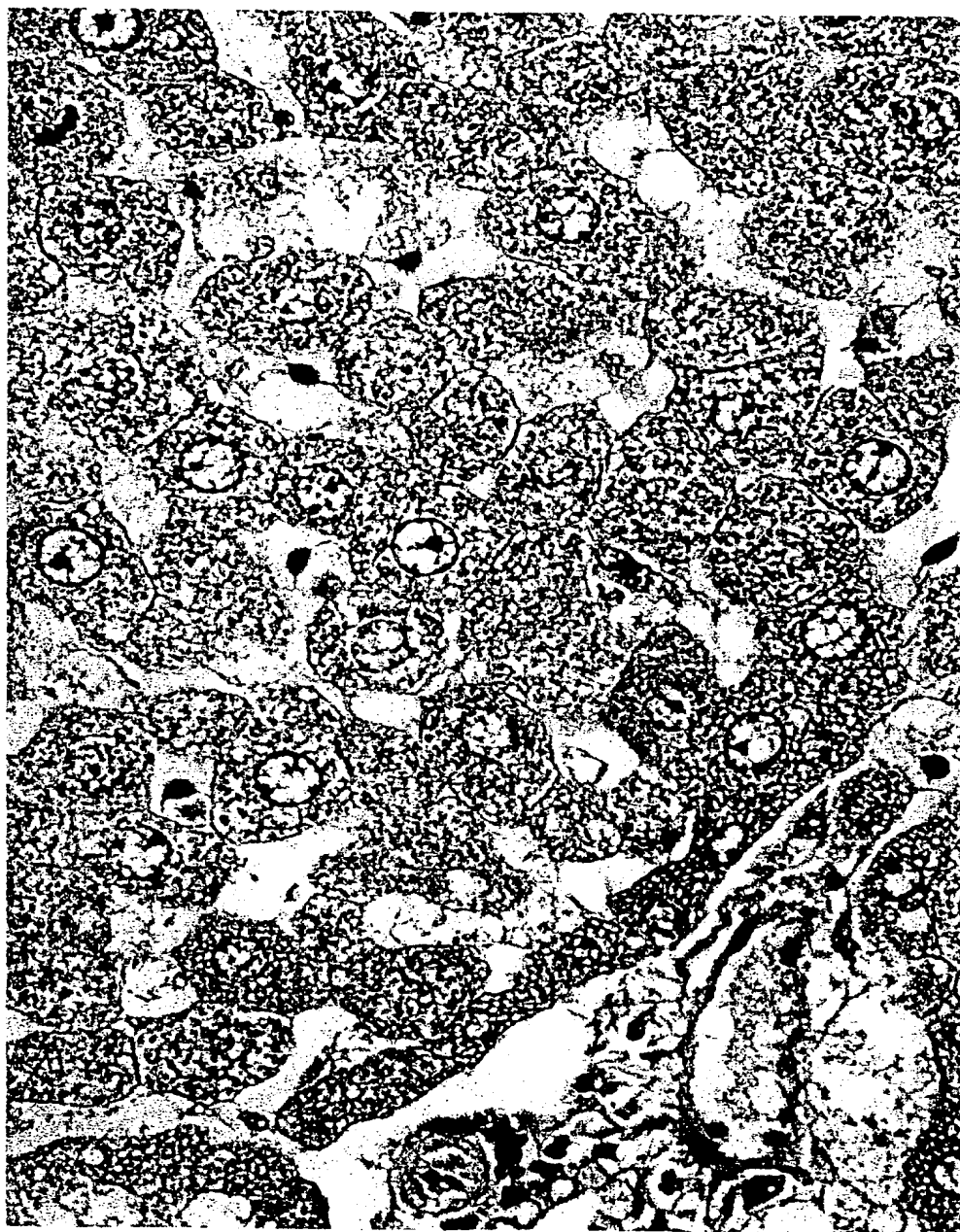


Figure 3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 97/00176

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/785

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NUZHAT ZAMAN ET AL.: "Effects of cholestyramine and parenteral nutrition on hepatic metabolism of lidocaine: a study using isolated rat liver perfusion" JOURNAL OF PARENTERAL AND ENTERAL NUTRITION, vol. 20, no. 5, September 1996, USA, pages 349-356, XP002045626 see page 349 - page 350, column 1 see page 353, discussion, 1st. paragraph see page 355, last paragraph	1-6
Y	---	16-25
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

4 November 1997

Date of mailing of the international search report

20.11.97

Name and mailing address of the ISA

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Authorized officer

Alvarez Alvarez, C

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/CA 97/00176

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 814 354 A (ISAAC GHEBRE-SELLASSIE ET AL.) 21 March 1989 cited in the application see column 1, line 5 - line 14 see column 3, line 33 - line 46	7,8,10, 12
Y	---	13-25
Y	GB 931 921 A (LABORATORIES ROQUES) 24 July 1963 see page 1, line 12 - line 16 see page 1, line 78 - line 81 see page 5, line 16 - line 22	13
Y	---	14
Y	WO 95 18608 A (N.V. NUTRICIA ET AL.) 13 July 1995 see claims 1,2,5	15
Y	EP 0 490 561 A (EFAMOL HOLDINGS PLC) 17 June 1992 see claims 1-6 -----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 97/00176

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 18-25
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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